collaborators developed improved procedures for subfractionation of Golgi fractions, and Fleischer, Fleischer, and Ozawa (1969) localized galactosyltransferase in the Golgi fraction – the first enzyme to be primarily associated with the Golgi apparatus.

After contending that the Golgi apparatus was an artifact of osmium staining, and prohibiting discussion of it in the Rockefeller laboratory for a number of years, George Palade was led back to investigating the Golgi apparatus in the 1960s as an outgrowth of his biochemical research on protein synthesis in ribosomes discussed previously. Palade and Siekevitz, using $^{14}$C-leucine as a tracer, established a migration in the pancreatic exocrine cell of $\alpha$-chymotrypsinogen from the ribosome into the lumen of the endoplasmic reticulum and ultimately into zymogen granules which were excreted from the cell. Detailing the path and activities occurring during this migration now became a focus of research. In an initial study in Palade's laboratory, Lucien Caro (1961; see also Caro & Palade, 1964) used $^{3}$H-leucine, which appeared within three to five minutes of injection in the endoplasmic reticulum, and then after twenty to forty minutes in the condensing vacuoles on the *trans* side of the Golgi stack.[38] After an hour the label appeared in the zymogen granules. This was critical evidence in establishing the transport of secretory proteins from the ribosomes through the membranous system of the Golgi apparatus to the zymogen granules that would then be secreted.

These initial findings were further elaborated in a series of studies with graduate student James Jamieson employing tissue slices from guinea pigs beginning in 1966. They arrived at a more detailed characterization of the migration of membrane-bound vesicles from the endoplasmic reticulum through the components of the Golgi apparatus to discharge from the cell (Jamieson & Palade, 1966; Jamieson & Palade, 1967a; Jamieson & Palade, 1967b). Key to their work was the use of three-minute exposure to leucine-$^{14}$C, a radioactive amino acid ("labeling pulse"), followed by removal of the unincorporated label ("chase"), allowing for better time resolution of the radioactive material.

Jamieson and Palade demonstrated that proteins, after leaving the endoplasmic reticulum, are encapsulated in small peripheral vesicles on the *cis* side of the Golgi stack and appear (after about thirty minutes) in condensing vacuoles on the *trans* side of the stack. In the interval they presumably traveled through the Golgi stacks, although Jamieson and Palade do not focus on that

[38] At about the same time, Warshawsky, Leblond, and Droz (1961) used labeled leucine in pancreas cells to trace the sites of uptake and the path of migration. They, however, lacked the ability to examine the results with the electron microscope.